Interaction of Histone H1 with *cis*-Platinum Modified DNA

Elena G. Paneva[a], Nadejda C. Spassovska[a], Konstantin C. Grancharov[a], Jordanka S. Zlatanova[b] and Julia N. Yaneva[a]

[a] Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[b] Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331-7305, U.S.A.

Z. Naturforsch. **53c,** 135–138 (1998);
received November 13/December 18, 1997

cis-DDP, *trans*-DDP, Histone H1, DNA, Protein/DNA Complexes

Cis-diamminedichloroplatinum(II) (*cis*-DDP) is known as an effective anticancer drug. Its therapeutic effect is supposed to be a consequence of the covalent binding to DNA. A number of cellular proteins were found to bind selectively to DNA modified by *cis*-DDP (but not by its isomer *trans*-DDP). Here we present our observations on interaction of the linker histone H1 with *cis*- and *trans*-DDP modified DNA fragments. The results afford new experimental information about the preferential binding of histone H1 to *cis*-DDP-distorted DNAs *versus trans*-DDP modified ones.

Cis-diamminedichloroplatinum(II) (cisplatin or *cis*-DDP) is a potent chemotherapeutic agent widely used in clinical practice against several types of human malignancies. The therapeutic effect is believed to arise as a consequence of *cis*-DDP binding to DNA (Sherman and Lippard, 1987), but it cannot be explained solely on the basis of DNA binding, because a number of closely related compounds, among which the geometric isomer *trans*-DDP, are not effective although they also damage DNA. The differential biological effect of different Pt-compounds may lie in the differential processing of different Pt-DNA adducts by the cell. The realization that different adducts may be processed differently by the cell focused research on identifying proteins specifically interacting with *cis*-DDP-modified DNA.

cis-DDP and its biologically inactive stereo isomer *trans*-DDP form different types of adducts upon interaction with DNA (Table I). The main adducts of *cis*-DDPt are 1,2-intrastrand d(GpG) and d(ApG) cross-links in which the two chloride ions are replaced by the N7 atoms of guanine and adenine. A 1,3-intrastrand cross-link may also be formed, at a much lower frequency, at d(GpXpG), wehre X is any base. Other minor adducts may also arise, including interstrand cross-links involving guanine residues on opposite strands. The therapeutically inactive *trans*-DDP is incapable of forming the 1,2d(GpG) and d(ApG) adducts for stereochemical reasons, the two chloride ions being too far apart to be able to link neighbouring bases. It does form 1,3-intrastrand links and also cross-links opposite strands, but while *cis*-DDP reacts with guanine residues in d(GpC), the *trans*-isomer preferentially cross-links complementary G and C residues (see Table I). Biochemical and structural studies on Pt-damaged DNA reveal major distortions of the DNA double helix, including bending and unwinding (reviewed by Lilley, 1996).

Table I. Major adducts formed between *cis*- and *trans*-DDP and DNA *in vitro* (the number of + signs corresponds to the percentage of the specific cross-links out of the total cross-links formed) – according to Sherman and Lippard, 1987. Capital letters denote DNA bases representing targets for the action of platinum compounds: A – adenine, C – cytosine, G – guanine; X denotes any single base (adenine, cytosine, thymine, guanine).

Platinum compound	intrastrand d(GpG)	d(ApG)	d(GpXpG)	interstrand d(GpC) d(CpG)	d(G) d(C)
cis-DDP	+++	++	+	+	–
trans-DDP	–	–	+	–	+

Recent years have witnessed the discovery of a number of cellular proteins of still unidentified *in vivo* functions that recognize and bind selectively to *cis*platin-modified DNA. These include the chromatin nonhistone proteins HMG1 and HMG2, the human structure-specific recognition protein 1 (SSRP1), the yeast intrastrand cross-link recognition protein lxr1, human UBF. All these proteins belong to the HMG1-box protein family in that they contain one or multiple copies of a specific DNA-binding motif, the HMG1-box, first described in the abundant HMG2 proteins (Bustin and Reeves, 1996). The HMG-1 box is known to

Reprint requests to Dr. Julia Yaneva.
Fax: 00359-2-723507.
E-mail: Yanev@math.acad.bg

0939–5075/98/0100–0135 $ 06.00

bind to distorted DNA structures and bend and unwind DNA upon binding (Grosscheld, 1995).

Here we present evidence that another major chromatin protein, the linker histone H1, also binds preferentially to *cis*-Pt-modified DNA.

Based upon a number of existing similarities in the binding of HMG 1/2 and linker histones (H1, H5, and subtypes) to DNA (Zlatanova and Yaneva, 1991; Zlatanova and van Holde, 1996) we hypothesized that these histones may also preferentially bind to *cis*-platinated DNA. In order to check this we performed band-shift experiments with globally platinated DNA fragments, obtained by restriction endonuclease digestions of plasmid DNAs. Such globally modified DNA fragments form a heterogenous population of molecules with different number of sites of modification.

To determine the correct ratio of DDP per nucleotide needed to reach a saturation level of modification, plasmid pBR322 codigested with restriction endonuclease HincII/EcoRV, was titrated with increasing amounts of either the *cis*- or *trans*-isomer. The degree of modification was monitored by the retardation of the electrophoretic mobility of DNA, caused by the Pt-cross-linking (Fig. 1). The retardation levelled off at about 0.025 DDP/nucleotide, and this ratio was chosen for further experiments.

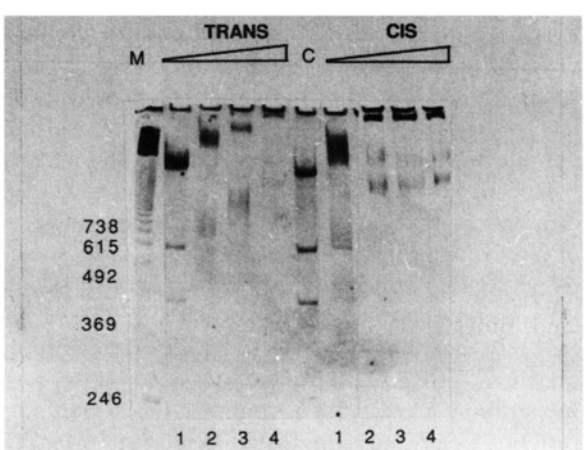

Fig. 1. Titration of plasmid pBR322 codigested with HincII/EcoRV with increasing amounts of *cis*- or *trans*-DDP. M denotes marker 123 bp ladder DNA (Gibco BRL, Life Technologies, U.S.A.). C denotes control, unmodified DNA. Lanes 1, 2, 3, and 4 in each case designate DDP/nucleotide molar ratio of 0.0025, 0.025, 0.25, and 2.5, respectively.

The first set of experiments on histone H1 binding was performed with a HincII/EcoRV digest of plasmid pBR322. The population of restriction fragments was modified with either *cis*- or *trans*-DDP; the unmodified digest was used as a control. The differently modified digests were titrated with increasing amounts of histone H1 and analyzed by electrophoresis on polyacrylamide gels. Because the interaction of DNA with highly positively charged histone H1, the movement of DNA fragments in the electrical field is retarded or fully stopped (in the cases of the formation of insoluble protein/DNA complexes). The results (Fig. 2A) demonstrate that a significant decrease in the amount of unmodified DNA restriction fragments as a result of histone H1 binding was first observed at one molecule of histone H1 per 32 bp of DNA. In contrast, much less histone H1 was needed to obtain a similar degree of complex formation in case of both the *cis*- and the *trans*-modified fragments. The preference of histone H1 to *cis*-modified DNA is especially well pronounced, since the first visible changes in the amount of soluble DNA fragments appear at 1 molecule of H1 per 160 bp; the fragments are completely complexed with the histone at one molecule of H1 per 64 bp DNA. The formation of insoluble complexes between the histone and the DNA fragments was not unexpected, in view of the presence of two DNA-binding sites in the globular domain of the protein, which will facilitate the formation of network aggregates (Ramakrishnan *et al.*, 1993).

When a HinfI restriction digest of plasmid pUC19 was titrated with increasing amounts of histone H1, and then run on polyacrylamide gels, the electrophoretic patterns obtained with the modified and unmodified fragments again differed considerably (Fig. 2B). With the unmodified control digest, the DNA fragments persisted in the gel even at the highest H1/DNA ratio tested, although the fragments were gradually shifted to higher positions in the gel, reflecting the binding of histone H1 to DNA. The *cis*-platinated fragments, on the other hand, disappeared from the gel, forming insoluble H1/DNA complexes, even at a ratio as small as one molecule of H1 per 25 bp. The electrophoretic behaviour of the *trans*-modified fragments was intermediate between that of unmodified and the *cis*-platinated frag-

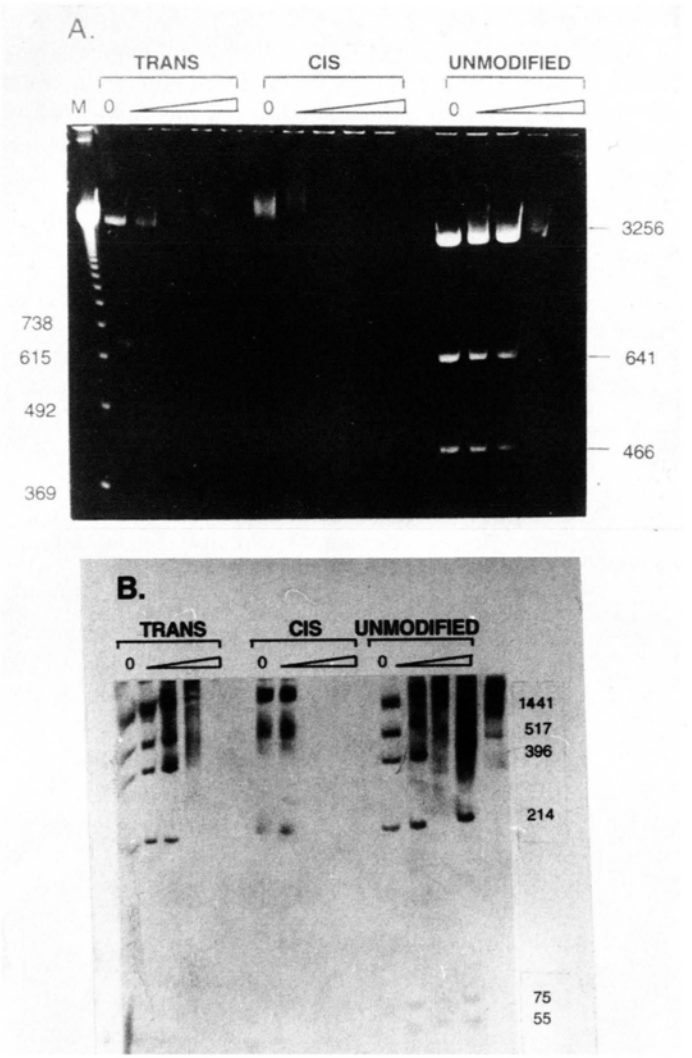

Fig. 2. Titration of three different sets of restriction fragments – unmodified, *cis*- or *trans*-DDP modified, as denoted on the figure – with increasing amounts of histone H1. DNA was modified at a 0.025 DDP/nucleotide molar ratio. The ratio of histone H1 to DNA in successive lanes was 1/160, 1/64, 1/46, and 1/32, expressed as a molecule of histone H1 per number of base pairs of DNA. (A) plasmid pBR322 codigested with Hinc II/Eco RV; (B) plasmid pUC19 digested with Hinfl restrictase. The gel in (A) is stained with ethidium bromide, that in (B) is silver stained.

ments, again, pointing out to a preference of binding of the histone to the *cis*-platinated DNA.

The results obtained in this initial study show that histone H1 binds preferentially to DNA fragments modified with the therapeutically active *cis*-DDP. Further experiments will be needed to quantify the degree of preference and to try to understand whether it has some relevance to the biological activity of *cis*-DDP.

Experimental

Bacterial plasmids pBR322 and pUC19 were isolated and purified using standard procedures (Sambrock *et al.*, 1989). Digestions with restriction endonucleases are performed following the recommendations of the manufacturer (New England, BioLabs, U.S.A.). The purity of DNA preparations was checked on 1% agarose gels.

For the modification of DNA fragments with platinum compounds DNA restriction fragments were incubated at 37 °C overnight with either *cis*- or *trans*-DDP (see Table I) in TE to various DDP/nucleotide molar ratios (Sherman and Lippard, 1987). The isomers of DDP were synthesized according to Spassovska *et al.*, 1981. Damaged DNA was freed from unbound DDP by ethanol precipitation.

Histone H1 was isolated from mouse liver nuclei by high saltextraction and purified by ion exchange chromatography on CM Sephadex C25 (Banchev *et al.*, 1991). The purity of the samples was verified by 15% polyacrylamide gel electrophoresis under denaturing conditions (Sambrook *et al.*, 1989).

Histone H1/DNA complexes were formed in 15 µl of binding buffer (10 mM Tris-(hydroxymethyl)aminomethane, pH 8.0, 20 mM NaCl, 0.1 mM PMSF) containing 0.2 µg of DNA and increasing amounts of histone H1. Incubations were carried out for 20 min at room temperature. Complexes formed were electrophoresed on 6% polyacrylamide gels (AA:bisAA, 29:1) in Tris-acetate/EDTA buffer (pH 7.5) at 15 V/cm. DNA was visualized by ethidium bromide or silver staining. Gels were photographed using black/white No. 667 Polaroid positive films. Pictures of silver-stained gels were taken using white black illumination.

Acknowledgements

This research was funded by National Science Fund (NSF No. K-529/95) to JY and Fogarty International Research Collaboration Award (FIRCA TW00568-02) to Kensal van Holde and JZ.

Banchev T., Srebreva L. and Zlatanova J. (1991), Purification of histone H1° and its subfractions under non-denaturing conditions. Biochim. Biophys. Acta **1073,** 230–232.

Bustin M. and Reeves R. (1996), High mobility group chromosomal proteins: architectural components that facilitate chromatin function. Progr. Nucl. Acids Res. Mol. Biol. **54,** 35–100.

Grosscheld R. (1995), Higher-order nucleoprotein complexes in transcription: analogies with site-specific recombination. Curr. Opin. Cell Biol. **7,** 362–370.

Lilley D. M. J. (1996), Cisplatin adducts in DNA: distortion and recognition. JBIC **1,** 189–191.

Ramakrishnan V., Finch J. T., Graziano V., Lee P. L. and Sweet R. M. (1993), Crystal structure of globular domain of histone H5 and its implications for nucleosome binding. Nature **362,** 219–223.

Sambrook J., Fritsch E. F. and Maniatis T. (1989), Molecular Cloning. A Laboratory Manual. Second Edition. Cold Spring Harbor Laboratory Press.

Sherman S. E. and Lippard S. J. (1987), Structural aspects of platinum anticancer drug interactions with DNA. Chem. Rev. **86,** 1153–1181.

Spassovska N. C., Bonchev P. R., Grancharov K. C. and Golovinsky E. V. (1981), Method for synthesis of *cis*-diamminedichloroplatinum(II). Bulgarian Patent No. 54667.

Zlatanova J. and van Holde K. (1996), The linker histones and chromatin structure: new twists. Progr. Nucl. Acids Res. Mol. Biol. **52,** 217–259.

Zlatanova J. and Yaneva J. (1991), Histone H1/DNA interactions and their relations to chromatin structure and function. DNA Cell Biol. **10,** 239–248.